No. 4

PAPERS READ AT A SYMPOSIUM ON HUMIC ACID HELD IN UNIVERSITY COLLEGE, DUBLIN, ON SEPTEMBER 10th & 11th, 1959

(Published July 22, 1960)

No. 4 A

THE NATURE AND DISTRIBUTION OF HUMIC ACID

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There has been considerable confusion regarding the nomenclature of the organic matter in the soil. The most commonly accepted terminology used at present is to restrict the term humus to the amorphous dark brown or black organic material as distinct from the partially decomposed plant and animal remains. Humus is further sub-divided into (a) humin, which is insoluble in sodium hydroxide; (b) humic acid, soluble in sodium hydroxide, precipitated by hydrochloric acid, and insoluble in alcohol; (c) fulvic acid, soluble in sodium hydroxide, not precipitated by hydrochloric acid; (d) hymatomelanic acid soluble in sodium hydroxide, precipitated by hydrochloric acid and soluble in alcohol. All of these occur in soils and composts but the proportions vary in different soil types. The available evidence suggests that the humic acid fraction represents either a single chemical substance or a group of closely related substances. There are good reasons for believing that humic acid is primarily non-nitrogenous and that the presence of nitrogen in many preparations is due to secondary combination with amino-acids or protein, with quinone groups in the main molecule. In the soil, humic acid normally occurs as a metallic humic acid complex, the metal varying with the soil conditions.

Interest in the chemical nature of the dark-coloured material in soil is of very long standing, and many of the most celebrated chemists of the last hundred years have attempted, at one time or another, to elucidate the structure of the substances concerned. Despite the amount of work already carried out, we have surprisingly little real knowledge of the basic structure of the chemical entities which are grouped under the general term, humus. Considerable confusion exists because of the heterogeneous nature of the soil organic matter. No attempt will be made to outline the historical development of the chemical investigation of humus. This has been done very fully by Waksman (1936), Tyurin (1937), and Bremner (1951, 1954).

When organic debris from plants and animals falls to the ground, micro-organisms and small animals begin to attack and decompose the fallen material. The organic matter in the soil at any one time, therefore, consists of a mixture of plant and animal fragments in various stages of decomposition, the micro-organisms and their decomposing remains, and the small animals which have often converted a considerable proportion of the original organic material into faecal pellets.

It is important to distinguish between the true amorphous humus and the organic material which still possesses recognizable cellular structure. Most workers would restrict the term humus to the dark-coloured amorphous material, and would exclude the mucilaginous colourless polyuronides which appear to be products of bacterial or fungal synthesis. Nevertheless, some investigators consider that dark colour is not necessarily a characteristic of humus. Estimation of the total humus material can be accomplished by removing the partially decomposed plant material with acetyl bromide, and estimating the residual organic matter (Karrer, see Waksman, 1936, p. 81). Various methods employing acetyl bromide to remove the partially decomposed plant remains before extracting the humic substances have been widely used by continental workers.

The importance of the four main fractions in humus was stressed by Oden (1919), and although his terminology has been altered slightly, his divisions have been widely accepted. Prolonged extraction of soil or compost with alkali or ammonium hydroxide leaves an insoluble residue variously known as *humus coal, humin* or *ulmin*. Addition of mineral acid to the extract produces a precipitate. The humic material remaining in solution is the *fulvic acid* fraction. This corresponds to the *crenic* and *apocrenic* acids of Berzelius. The alcohol-soluble fraction of the precipitate is known as *hymatomelanic acid* (also spelt haematomelanic and hematomelanic) or ulmic acid, and the alcohol-insoluble fraction of the precipitate as *humic acid*. It seems highly desirable that the use of these terms should be restricted and not extended to include supposedly related substances. This is particularly important when dealing with model substances such as the humus-like material produced by caramelizing carbohydrates with acid. Some distinct name such as *saccharo-humic acid* should be used. It is unfortunate that the name fulvic acid has also been used for a mould product with no relation to soil fulvic acid.

Attempts to obtain purified preparations of these various fractions and to determine their elementary composition have been numerous, but inconclusive. Similar inconclusive and unsatisfactory results have been obtained in investigations of the molecular weights of the different components. In recent years the greater part of the chemical studies of humus have been concerned with the humic acid fraction. The properties of this fraction cannot be defined precisely, and there has been little advance over the general account given by Shmuk (1924). He regarded humic acid as a dark-coloured substance of plant or animal origin, obtained by extracting soil with alkali and precipitating with acid. He emphasized that some of its properties may be changed during the extraction. When alkaline extractants are used there is often an uptake of oxygen. In its chemical properties, he believed it to be a nitrogenous material with an acidic character. He associated the bulk of the protein in soil with the humus, but was vague on the method of combination. The nitrogenous material was essentially similar to protein decomposition products, but because glucosamine was not found, he assumed that the protein was not of microbial origin. The acidic properties were attributed partly to the colloidal nature of humic acid, as well as to carboxyl groups. Humic acid is capable of forming metallic salts, as well as absorption complexes. In addition to carboxyl groups, he believed the evidence was strong for the presence of benzyl nuclei with hydroxyl groups in the nuclei or side chains. He held the view that the naturally occurring humic acids were similar in structure to artificial humic acids prepared from carbohydrates or proteins.

In America and many other English-speaking countries, Waksman's (1936) view that humic acid is essentially a ligno-protein has been widely held. This view developed largely from studies of decomposing plant materials, particularly in composts. During the early stages of composting, the sugars, starches, hemicelluloses, and celluloses, decompose more rapidly than the lignins. At this stage there is little loss of nitrogen, and well-composted material contains a highly resistant dark residue which Waksman believed to be a ligno-protein complex. This view has been criticized by many workers, particularly on the continent (e.g. Kononova, 1951). The work of Tinsley and Maung (1956), who isolated what appears to be a genuine ligno-protein from soil, shows that such complexes do not form a major part of soil organic matter.

Most theories concerned with the formation of humic acid postulate a polymerization of polyphenolic units. Some workers regard these as having been derived from the decomposition of lignin residues, others believe the units are synthesized from simpler carbon compounds by micro-organisms. The isolation of substances indistinguishable from humic acids from micro-organisms grown on glucose and mineral salts (e.g. Lund *et al,* 1953) has led some workers to regard humic acid as a product of microbial synthesis. Such a point of view has been stressed recently by Kononova and Aleksandrova (1959). The work of Mayaudon and Simonart (1958) in which glucose marked with C^{14} was added to soil and the radio-activity detected later in the humic acid, supports the view that the humic acid may be synthesized from relatively simple substances in the soil.

Nearly all workers have regarded nitrogen as an essential component of humic acid. Swaby (1959), in Australia, has postulated an oxidation of the polyphenols to quinones, and a coupling of these with amino acids derived from proteins. Such theories clearly regard the nitrogen as forming a structural part of the complex molecule. Tyurin, however, took the view that, in some humic acids at least, the presence of nitrogen was to be regarded as due only to an inevitable admixture of proteins with humic acid, and "that humic acid *per se* as a specific compound is a nitrogen-free substance" (see Joffe, 1949, p. 201). Evidence will be presented below in support of this idea.

In the field, humic acid seems to exist as metallic salts or metallic complexes. Acidic soils such as podzols have the humic acid present mainly as the iron or perhaps the aluminium humate. In soils with a higher calcium content, e.g. a chernozem, it is there as a calcium humate. The nature of the metallic ion present alters both the colour and the physical structure of the humic acid in the soil.

Scheffer (1954) has investigated two forms of humic acid, the grey and brown humic acids. He separates them by electrolytic coagulation with 2N. NaCl at pH 7. The grey coagulates while the brown stays in solution. As the result of his work, he is inclined to the view that the brown humic acid is derived from carbohydrates, whereas the grey comes from proteins and phenols.

Humic acids extracted from peat, composts, and many soils, contain appreciable quantities of nitrogen, usually up to 4%. This amount, however, varies considerably, and in extracts from sphagnum peat there may be much less than 1%. Acid hydrolysis shows that the nitrogen is primarily present as amino-acids. Kononova and Aleksandrova (1959) record lysine, histidine, arginine, aspartic acid, glycocoll, serine, glutamic acid, threonine, alanine, tyrosine, valine, methionine phenylalanine, and leucine from the hydrolysate of humic acid from a chernozem. The amino-acids recorded are typical of what would be obtained from a protein hydrolysis.

In natural soils humic materials occur primarily in the upper layers. Soils such as podzols which have a marked litter layer on the surface, usually have a zone at the junction of the litter and mineral soil very rich in humic material. Up to 75% of the organic material in this zone may be humified. A well-marked humus podzol may also have an accumulation of humic material in the B_1 horizon. Here, the total organic material may be up to 5 or 6% by dry weight, and 75-80% of this can be extracted as humic acid. In chernozem types of soil there may be 10% of organic matter in the upper 10 cm of soil, and of this organic matter about 40% is humic acid. There are many reports concerning the relative proportions of humin, fulvic, hymatomelanic, and humic acids in different soil horizons. Despite some claims to the contrary, there is no consistent pattern of distribution, either in the profile or in different soils. All four fractions occur in most, if not all, soils. Perhaps if a standard method of estimation ~~were~~ was used on a wide range of soils, characteristic distributions might become evident. At present, however, the many different extraction methods used makes it difficult to correlate the results of different workers.

The ash content of humic acids varies considerably according to the method of extraction. Assuming that humic acid occurs as a metallic humate, extraction with NaOH or with chelating compounds, and subsequent precipitation with acids would be expected to give the free humic acid with no appreciable ash content. Usually it is difficult to get the ash below 2-3% in humic acid extracted from podzols and 1% in extracts from chernozems. Extracts from peats, however, may contain appreciably less.

Purified humic acid from the B_1 horizon of a podzol in Cheshire contained 1.8% of ash. Analysis of this, by Dr. Riley of Liverpool, showed the presence of 16.5% titanium oxide and 18.4% ferric oxide. Silica accounted for about 28%, and calcium and sodium between 5-10% each. These substances are fairly certainly contaminants, and humic acids prepared from other soils contained comparable amounts of silica and iron, but differed markedly in the other components.

It has been stated above that we regard our humic acid as primarily a nitrogen-free substance which is capable of combining with amino-nitrogen. This view is based on results obtained by my colleagues and me at Liverpool.

In experiments involving the extraction of humic acid from peat, composts, or many soils, there is considerable difficulty because of the very large amount of other organic matter present. For this reason we have chosen the B_1 horizon of a humus podzol as the source of our material. There are good reasons for believing that the humic acid is formed in the litter layer, and that it then migrates through the upper layers of the soil and accumulates at the top of the B_1 horizon, where it is deposited apparently in combination with iron, and perhaps aluminium. The greater part of the organic matter in this layer is humic material, the remainder being mainly a few small roots. The humic acid was extracted with either 50% or 100% lactic acid. The crude extract was purified by passing it through an amberlite (I.R. 120) column to remove cations, diluting, and then precipitating with hydrochloric acid. The precipitated humic acid was transferred to vinyl dialysis tubing, and dialysed against 20% HCl until iron could no longer be detected, and then against distilled water until chloride ions were no longer present. The humic acid was dried in a vacuum oven at 40°C. 1000 g fresh soil yielded 20 g of dried humic acid.

In other experiments the soil was extracted with 100% lactic acid, and the humic acid precipitated by adding ether. The precipitate was easily filtered off and washed with acetone, water, and methanol to remove free lactic acid and lactates. The product obtained in this way was regarded as the natural metal humate.

Studies carried out by Dr. Dean and Dr. Taylor have shown that this has the composition C, 44.1; H, 4.4; N, 0.6; Ash, 7.0; O Me 1.3%. The free acid has the composition C, 50.9; H, 4.1; O Me 1.2; N, 0.7. Treatment of this material with ferrous and ferric ions in aqueous dimethylformamide converted the humic acid into a substance with the same properties as the original metal humate. Methylation and acetylation studies gave reason to believe that the humic acid contains 4-5% phenolic hydroxyl, 5-7% acidic hydroxyl (largely carboxylic acid), 2-3% alcoholic hydroxyl, and 3% quinonoid oxygen.

The small amount of nitrogen is, we believe, indicative that the essential structure of humic acid does not contain nitrogen. Studies undertaken by Miss P. Latter show that if the nitrogen-poor humic acid is added to culture solutions in which certain soil fungi are growing, a humic acid preparation can subsequently be extracted containing approximately 4% of nitrogen, probably in the form of amino-acids. This high-nitrogen humic acid is very similar, e.g. in its electrophoretic properties, to the original nitrogen-poor humic acid.

Four per cent. of nitrogen seems to be about the maximum content found in humic acid, and may represent a saturation level. The wide range of nitrogen contents recorded from naturally occurring humic acids is possibly indicative of different levels of saturation.

A number of attempts were made to determine the purity and uniformity of the humic acid by electrophoretic studies. The major part of the material ran as a single well-defined band, with quite a high mobility. Subsequent experiments with a continuous flow equipment, gave evidence of some separation, but examination of the infra-red spectra of the material in the different bands has given no evidence of difference in structure. Experimental work now in progress suggests that the apparent separation into components is due, at least in part, to the physical heterogeneity of the material. If, for instance, the leading band of the humic acid in an electrophoresis experiment is collected and run again immediately it shows only slight spread, and moves as a single band. If, however, the humic acid is precipitated, dried, and redissolved, and then run again, there is a considerable spread approaching that originally found. Infra-red spectra of samples obtained in such experiments, show no evidence of difference in chemical structure between the different fractions.

In preparing this paper I have drawn freely on the knowledge and results of my colleagues, particularly Dr. Dean and Dr. Taylor from the Department of Organic Chemistry, and Miss Latter of the Department of Botany. Detailed accounts of their work will be published subsequently.

We wish to acknowledge gratefully the generous help given to our work by the Nuffield Foundation.

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